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I, LEANNE MYNOTT, MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PR5890 for a patent by INTREAT PTY LIMITED as filed on 22 June 2001.

I further certify that pursuant to the provisions of Section 38(1) of the Patents Act 1990 a complete specification was filed on 17 January 2002 and it is an associated application to Provisional Application No. PR5890 and has been allocated No. 2002224664.

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Twelfth day of March 2007

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AUSTRALIA
Patents Act 1990
PROVISIONAL SPECIFICATION
FOR A PROVISIONAL PATENT

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Invention Title: **An Antibody Cancer Therapeutic**

The following statement is a description of this invention

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This invention relates to antibody-based treatments of cancers, especially those derived from epithelial cells and malignant lymphoma.

Part of the basis for the invention is found in research into the purinergic receptor $P2X_7$ in epithelial cells. It is known that P2X binding sites are present in these cells and it has been possible to detect the distribution of the P2X receptors using antibodies specific to each of the subtypes. Subtypes $P2X_1$ to $P2X_7$ have been identified.

The $P2X_7$ subtype is involved in apoptosis or programmed cell death in many cell types including epithelial cells. It is referred to as a cytolytic receptor capable of forming pores that enable the cell to be flooded with excess calcium rather than simply acting as a calcium channel. Adenosine triphosphate (ATP) can induce cytolysis in cells such as leukocytes including lymphocytes, thymocytes, macrophages and dendritic cells through the $P2X_7$ receptors expressed on the cell surface. $P2X_7$ receptors open channels through the cell membrane within a second. Continued application of ATP leads to the formation of a pore within a few tens of seconds that induces apoptosis.

This invention uses a $P2X_7$ subtype-specific antibody to specifically bind to non-functional $P2X_7$ receptors expressed on epithelial cells forming part of preneoplastic or neoplastic tissue and on any neoplastic cell expressing non-functional $P2X_7$ receptors. Thus the receptor is only bound when in a close-gated or non-functional conformation even though it may be normally expressed in the cell membranes.

It has now been found that, in patients with epithelial cell cancer such as prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder and vaginal cancer, and malignant lymphoma but not confined to these, the non-functional $P2X_7$ receptors can be bound by using an antibody directed against an epitope that undergoes a conformational change from the structure present in functional receptors. It has been found that the amino acid sequence of the non-functional receptors can be identical to the amino acid sequence of functional receptors so that the cause of the conformational change in the receptors relates to interaction of the receptors with ATP. The ATP molecules act as receptor agonists, so that when ATP is bound to the receptors, they are able to open a channel through the cell membrane for the flow of calcium ions. Non-functionality is therefore caused by a lack of appropriate binding of the ATP agonists to the receptors. If ATP binding to the receptors is disrupted, the receptor conformation is altered and this can be treated

using an antibody specially designed to bind to the region of the protein affected by the binding of the ATP.

The specific sequence involved in the conformational change includes Pro210 in human $P2X_7$ receptors that undergoes a change in configuration from trans form to cis form in the absence of bound ATP. Thus an appropriate epitope sequence against which an antibody must be raised includes Pro210 and may extend either side of this residue to an appropriate extent necessary to induce an antibody response. This may include by way of example a segment extending from Gly200-Thr215 but is not confined to this segment.

Because current studies and investigations may not fully explain the working of the invention, it is necessary to define the invention in a number of aspects, as set out below. It is possible and likely that there will be overlap of at least some of those aspects.

Accordingly, in a first aspect, the invention provides an antibody to bind to cells in cancer and/or preneoplastic tissue, including those cancers derived in epithelial cells listed above, the antibody specially adapted to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors. The antibody may be polyclonal, monoclonal, recombinant or a humanised antibody or appropriate fragment and is preferably directed against an epitope located in the extracellular domain adjacent to the ATP binding sites and incorporating the proline at amino acid 210 in the human $P2X_7$ sequence that undergoes cis/trans isomerisation, with the cis conformer associated with the non-functional conformation.

In a second aspect, the invention provides for the use of the antibody of the invention as a therapeutic vehicle for treatment of cancer in a patient to regulate programmed cell death by targeting the aberrant or non-functional $P2X_7$ receptors expressed on the surface of the cells while leaving all cells expressing normal receptors untouched.

In a third aspect, the invention provides a pharmaceutical composition for treatment or prevention of disease or medical conditions, including cancers in a patient, the composition including a pharmaceutically effective amount of an antibody capable of regulating programmed cell death of cells having expressed on their surface aberrant or non-functional $P2X_7$ receptors.

The invention also provides a method of treating or preventing cancer or medical conditions in a patient, comprising administering to the patient a pharmaceutical composition as defined in the broad aspect above.

The invention also provides the use of a pharmaceutical composition defined in the broad aspect above, in the treatment or prevention of disease or medical conditions, including epithelial cell derived cancers and malignant lymphoma, in a patient.

The pattern of use of one or more of the above pharmaceutically effective compositions may need to be altered for optimum effect.

It will be apparent to those skilled in the art that many obvious modifications and variations may be made to the embodiments described herein without departing from the spirit or the scope of the invention.

Dated this 22nd day of June 2001

Intreat Pty Limited

by its Patent Attorneys

Chrysiliou Law